

APPENDIX

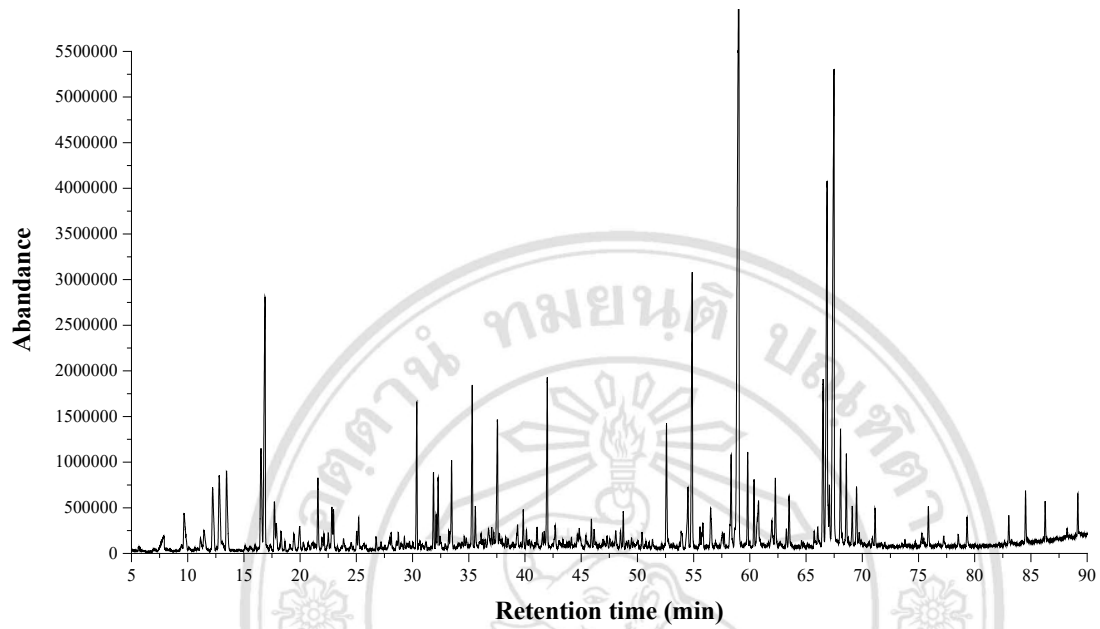


Figure A.1 Total ion chromatogram of fresh leaves

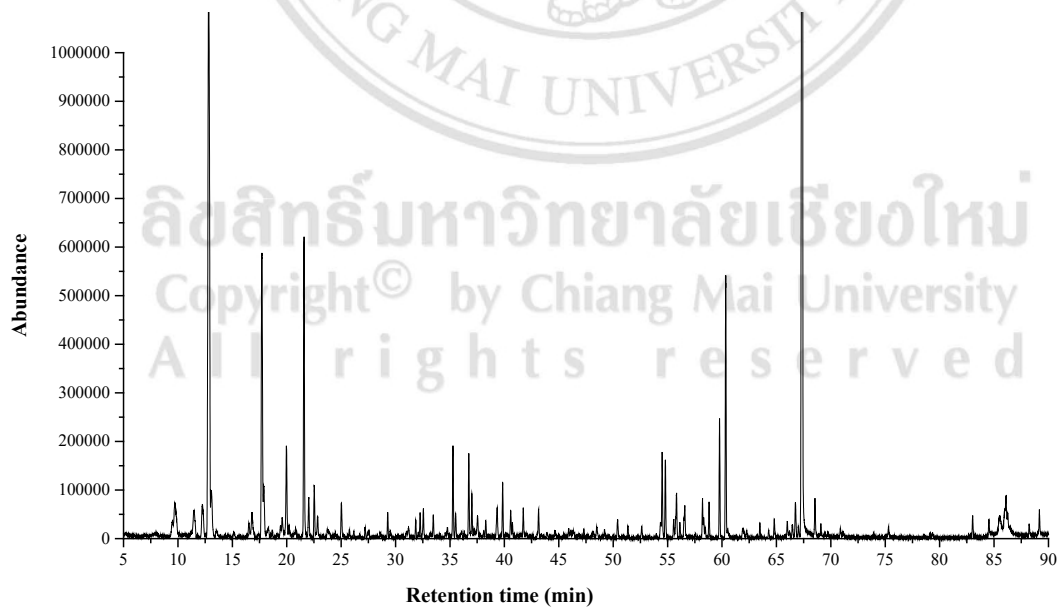


Figure A.2 Total ion chromatogram of steamed leaves

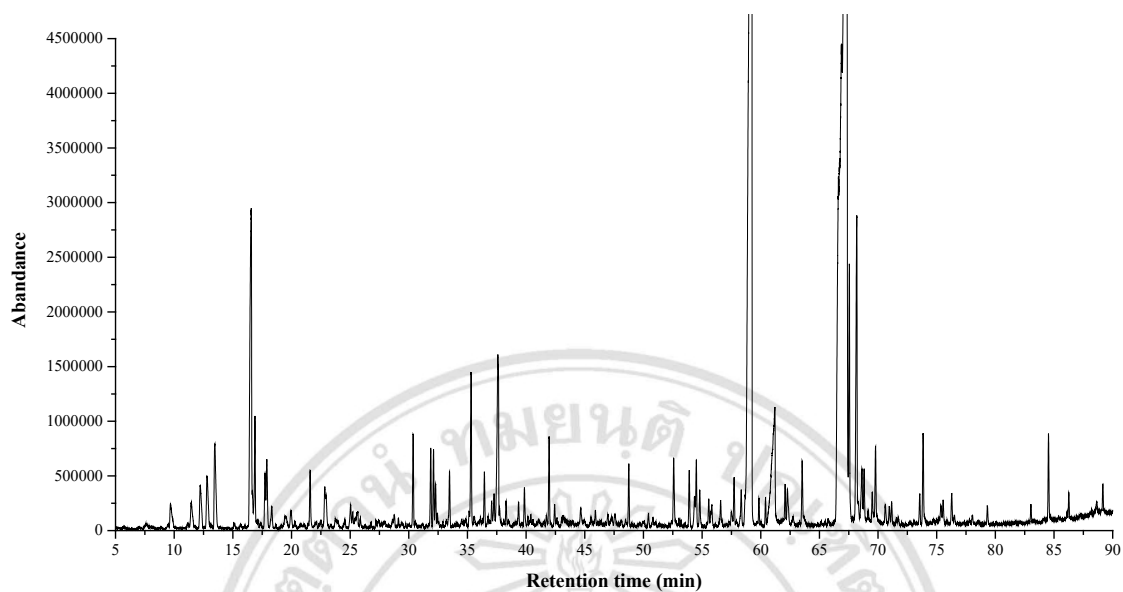


Figure A.3 Total ion chromatogram of 15 days fermented leaves

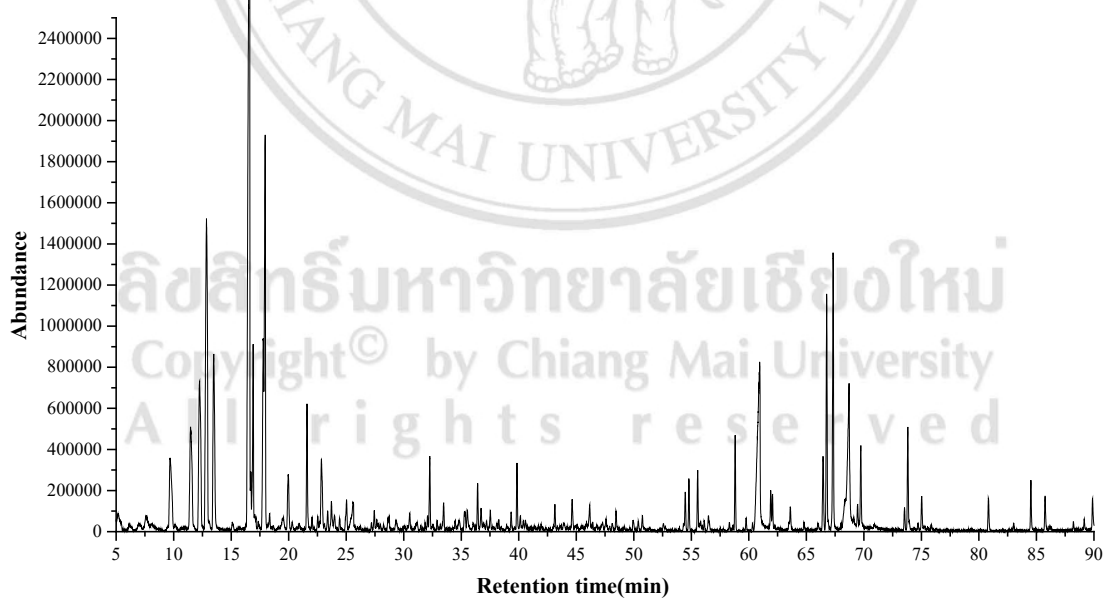


Figure A.4 Total ion chromatogram of 30 days fermented leaves

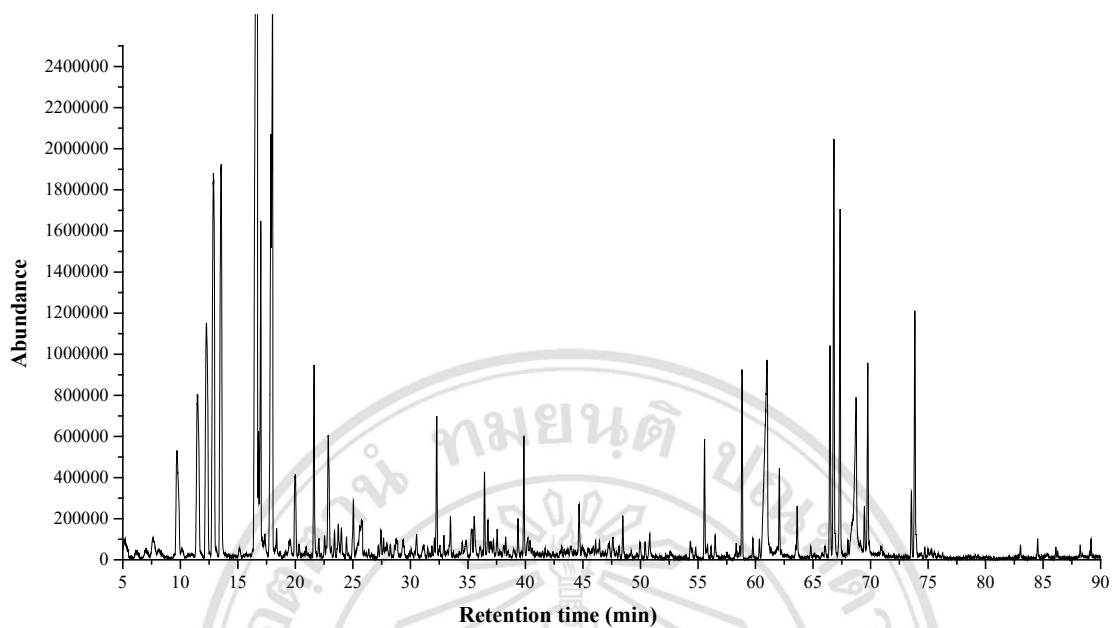


Figure A.5 Total ion chromatogram of 45 days fermented leaves

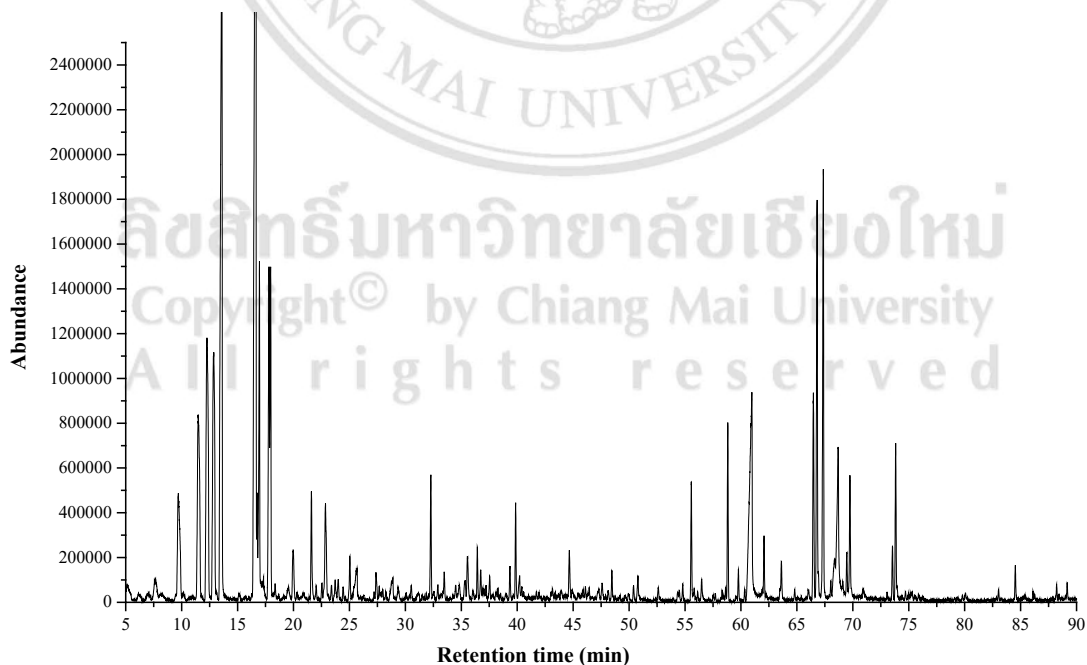


Figure A.6 Total ion chromatogram of 60 days fermented leaves

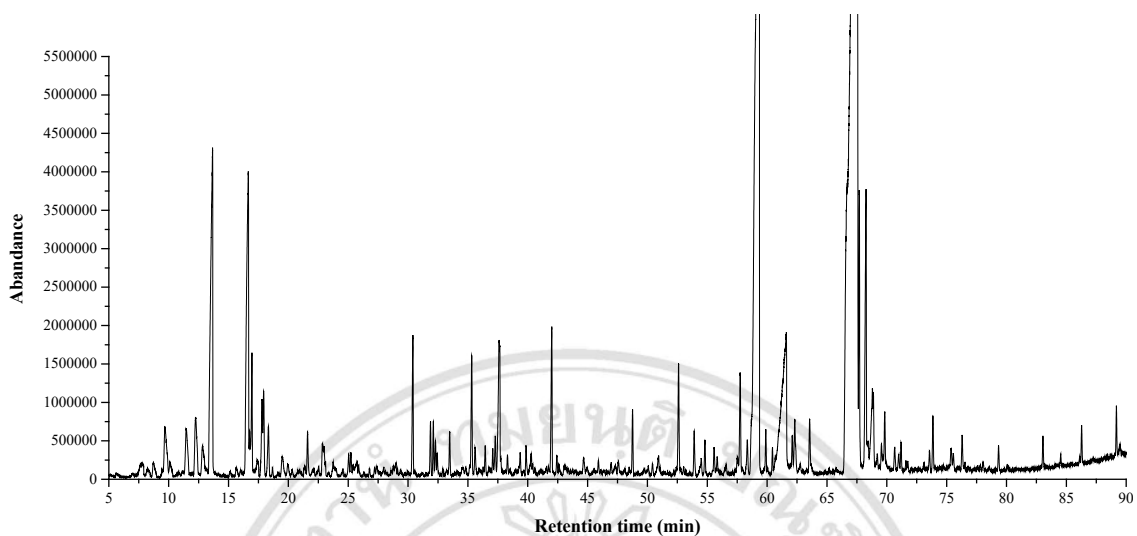


Figure A.7 Total ion chromatogram of 90 days fermented leaves

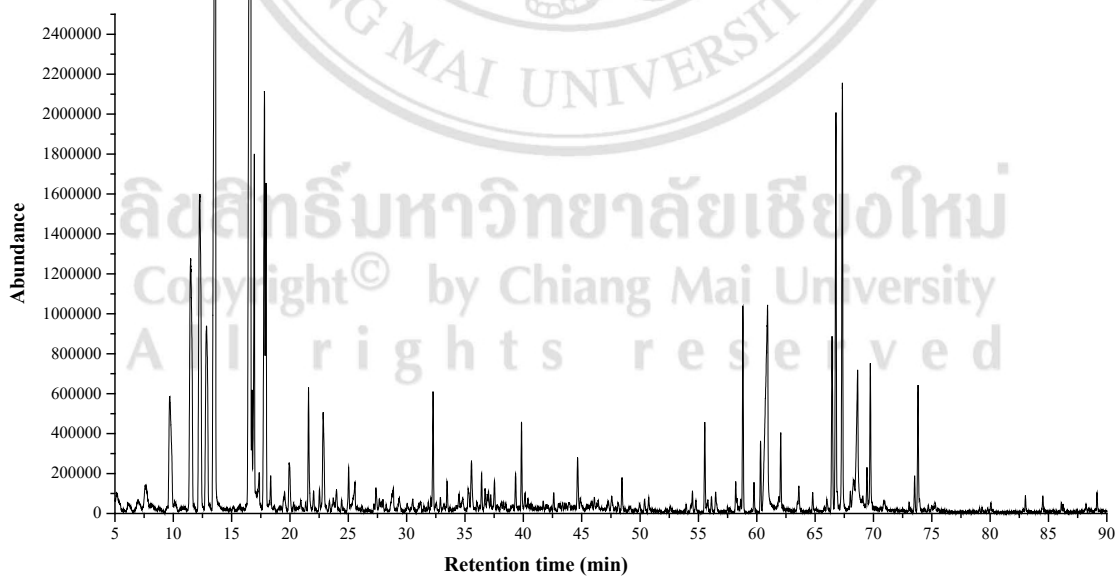


Figure A.8 Total ion chromatogram of 120 days fermented leaves

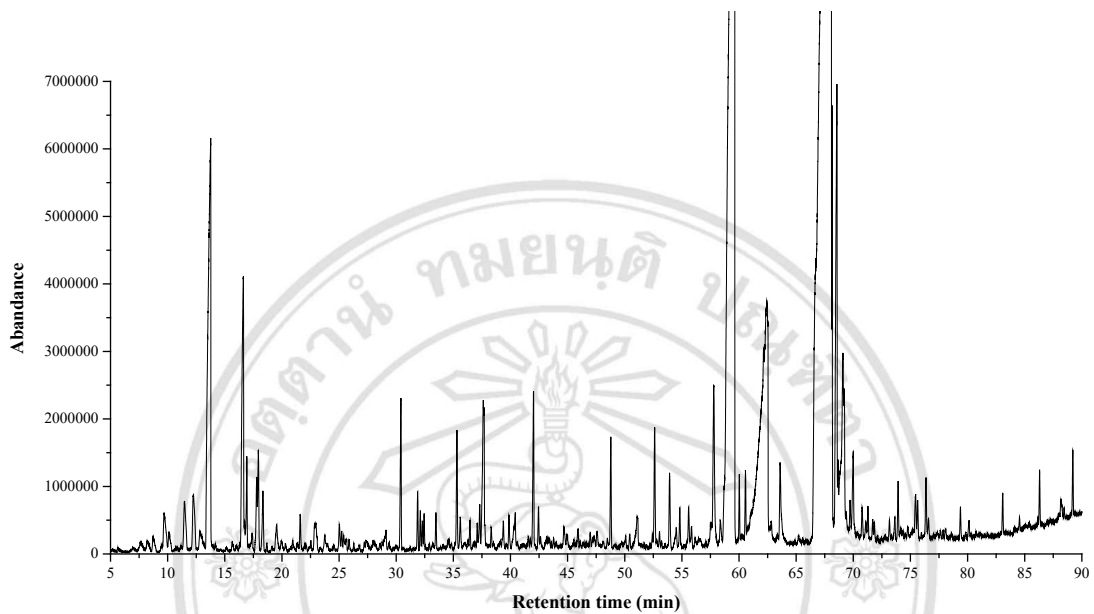


Figure A.9 Total ion chromatogram of 150 days fermented leaves

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Calculation of the detection limit⁸⁹

The detection limit was calculated from the linear regression line of the calibration curve. It was determined as follows:

$$Y = a + bx \quad (1)$$

where Y = Instrument signals
 x = concentrations
 a = intercept
 b = slope of the straight line

$$Y_L = Y_B + kS_B \quad (2)$$

$S_{y/x}$ can be calculated from the equation

where

Y_L = lowest detectable instruments signals

Y_B = Y intercept, a

K = constant depending on definition such as

$k = 1, 5, 3$ of 10 according to IUPAC, in calculation of limit of detection, $k = 3$ was used in this work

S_B = blank signal standard deviation

$$S_{y/x} = \sqrt{\frac{\sum (Y_i - \hat{Y})^2}{n-2}} \quad (3)$$

where

Y_i = response value from instrument corresponding to the individual x value

\hat{Y}_i = value of y on the instrument corresponding to the individual x value

n = number of point on the calibration line

From equation 1 and 2

$$Y_L = a + 3 S_{y/x} \quad (4)$$

$$Y_L = a + b C_L \quad (5)$$

Thus,

$$a + 3 S_{y/x} = a + b C_L$$

$$C_L = 3 S_{y/x} / b \quad (6)$$

The values lower than LOD are to be equated as called non-detected.

For example, from data of the gallic acid calibration curve with the concentration range 0.6-1.5 ppm:

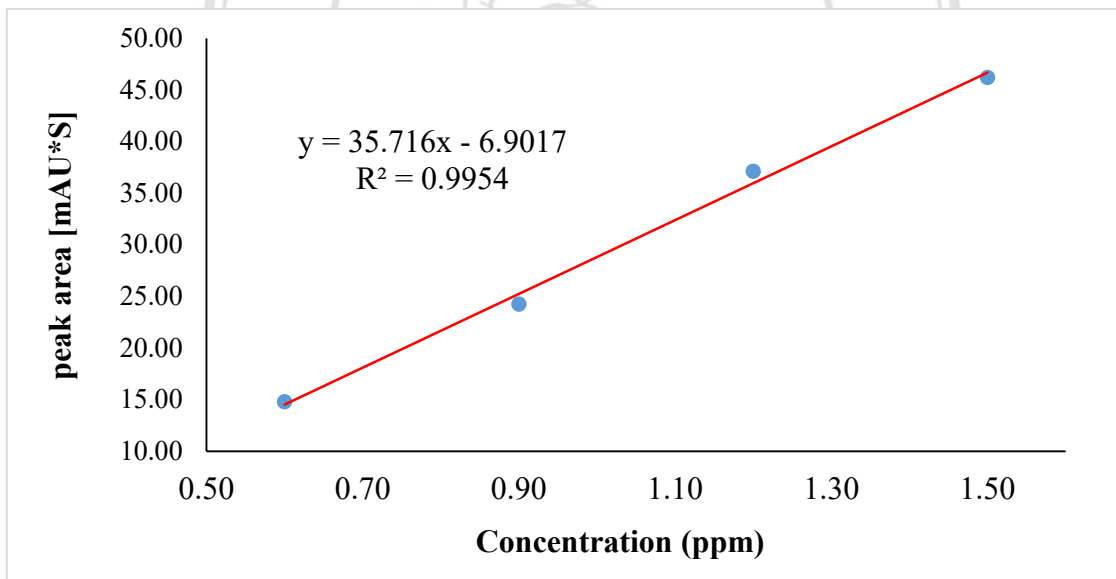


Figure A.10 Calibration curve of gallic acid

Linear regression of Figure A. 10; $y = 35.716x - 6.9017$

$$R^2 = 0.9954$$

Table A.1 The data used for calculation of the detection limit of gallic acid

	ppm (Xi)	Signal (Yi)	$X_i - \bar{X}$	$Y_i - \bar{Y}$	$(X_i - \bar{X})(Y_i - \bar{Y})$	$(X_i - \bar{X})^2$	\hat{Y}_i	$Y_i - \hat{Y}_i$	$(Y_i - \hat{Y}_i)^2$
	0.6	13.76	-0.45	-13.01	5.86	0.20	12.26	1.50	2.25
	0.9	20.23	-0.15	-6.54	0.98	0.02	21.93	-1.70	2.89
	1.2	30.51	0.15	3.74	0.56	0.02	31.61	-1.10	1.20
	1.5	42.58	0.45	15.81	7.11	0.20	41.28	1.30	1.69
Sum	4.2	107.09			14.51	0.45			8.03
Average	1.05	26.77							

By using equations 5 and 6

$$S_{y/x} = \sqrt{\frac{\sum (Y_i - \hat{Y}_i)^2}{n-2}}$$

$$S_{y/x} = \sqrt{\frac{8.03}{4-2}}$$

$$S_{y/x} = 2.00$$

and

$$C_L = 3 S_{y/x} / b$$

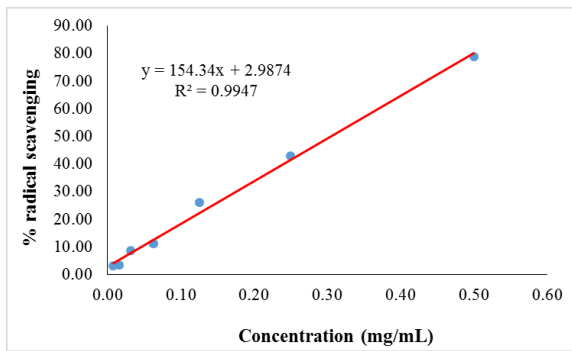
$$C_L = 3 (2) / 35.716$$

$$C_L = 0.19$$

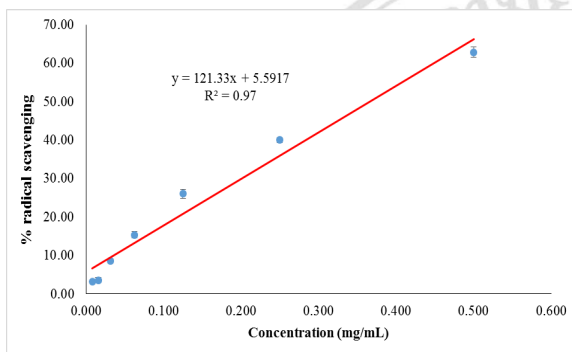
∴ Detection limit of gallic acid is 0.19 ppm

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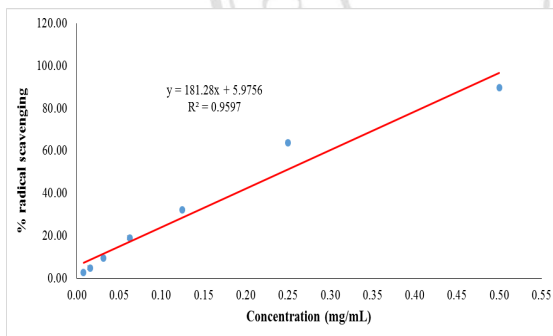
Calibration curve for IC₅₀ calculation



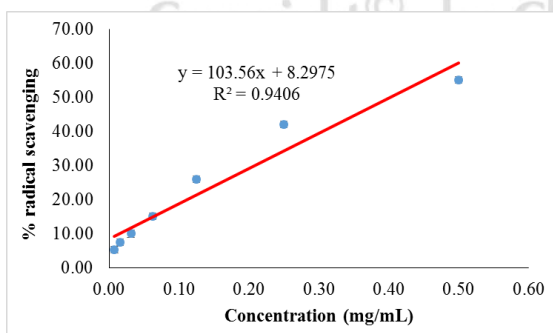
A



B

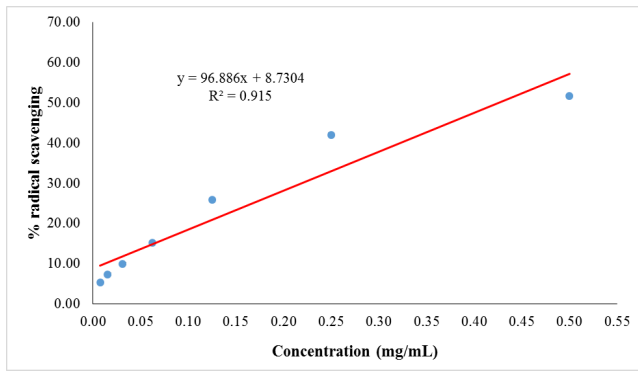


C

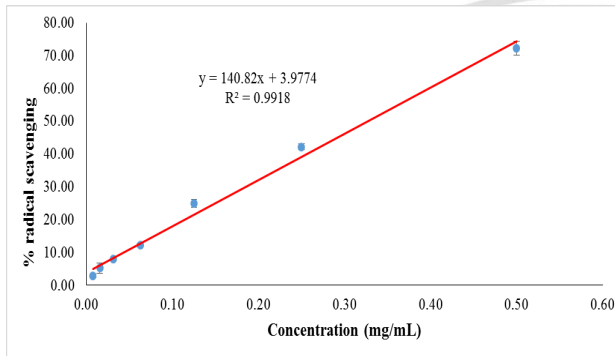


D

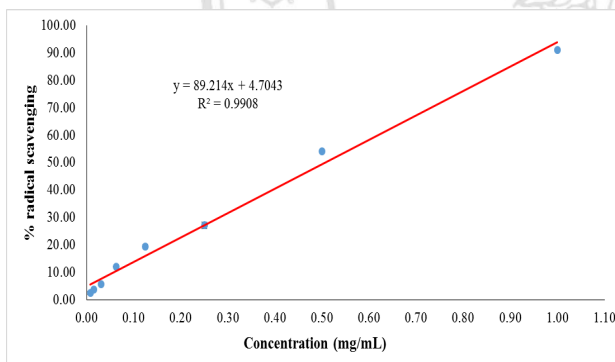
Figure A.11 Calibration curve of acetone extract; A: 25% aq., B: 50% aq., C: 80% aq. and D: 100%



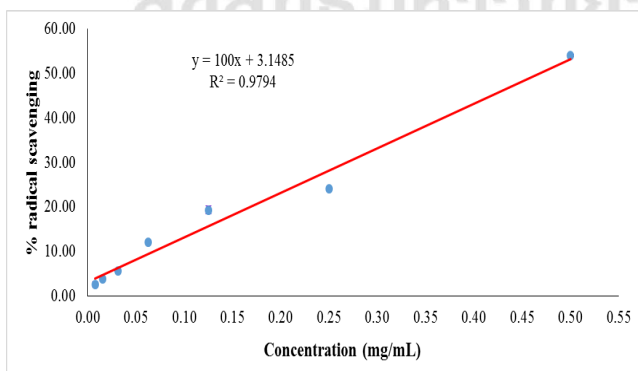
A



B

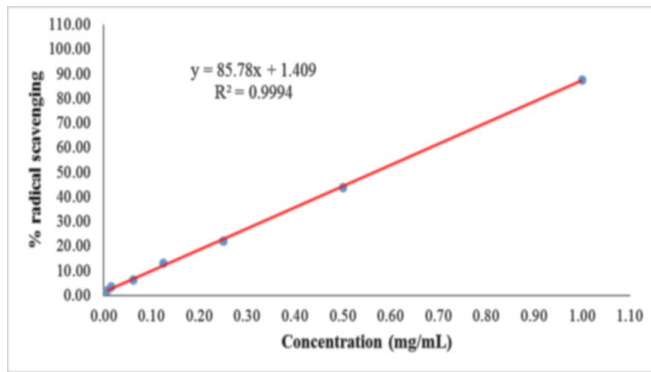


C

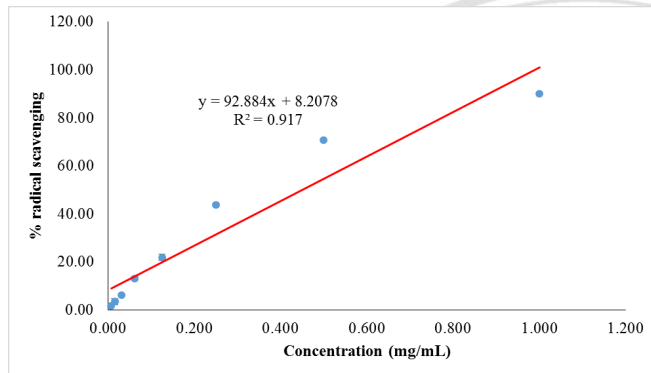


D

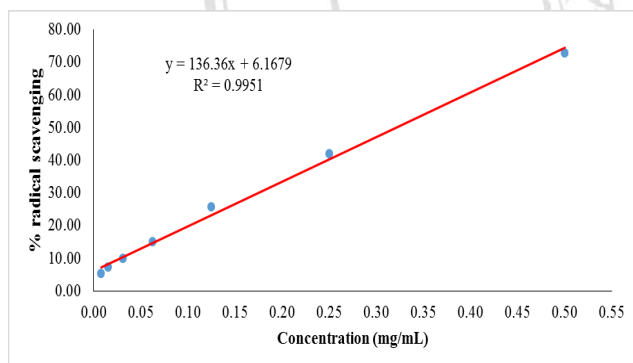
Figure A.12 Calibration curve of ethanol extract; A: 25% aq., B: 50% aq., C: 80% aq. and D: 100%



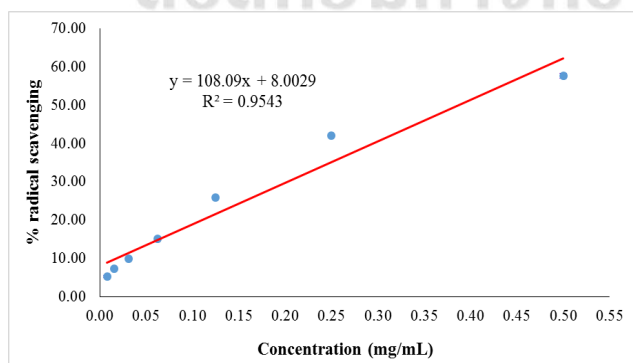
A



B



C



D

Figure A.13 Calibration curve of methanol extract; A: 25% aq., B: 50% aq., C: 80% aq. and D: 100%

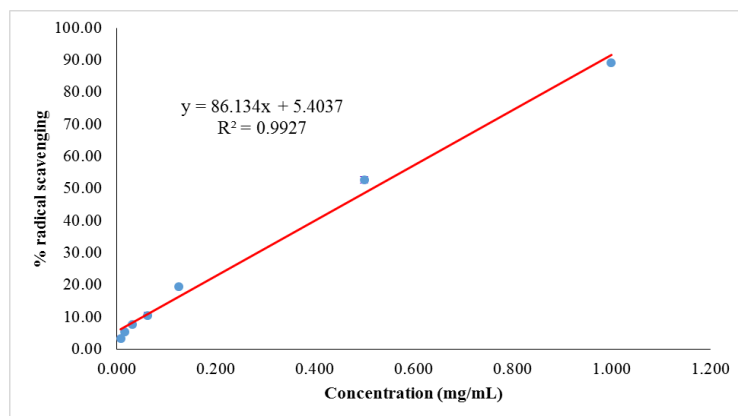


Figure A.14 Calibration curve of water

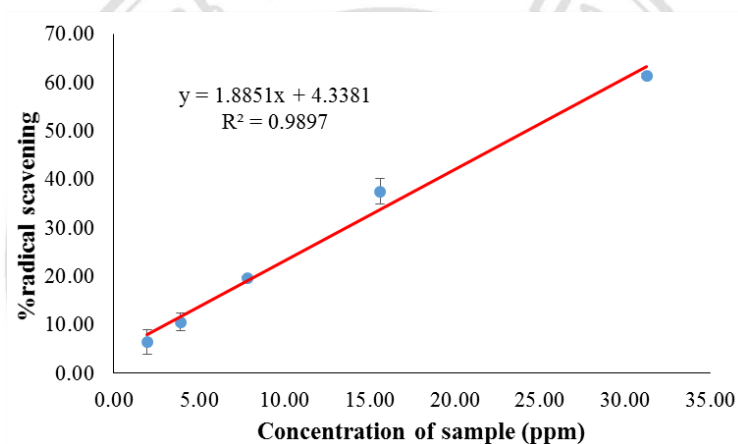


Figure A.15 Calibration curve of fresh leaves extract

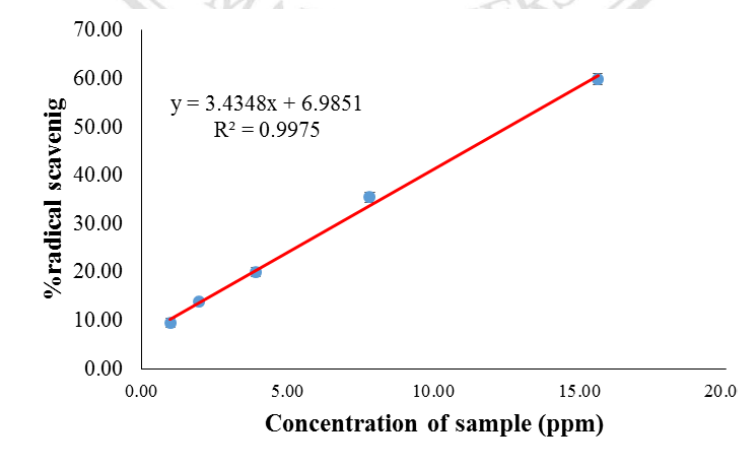


Figure A.16 Calibration curve of steamed leaves extract

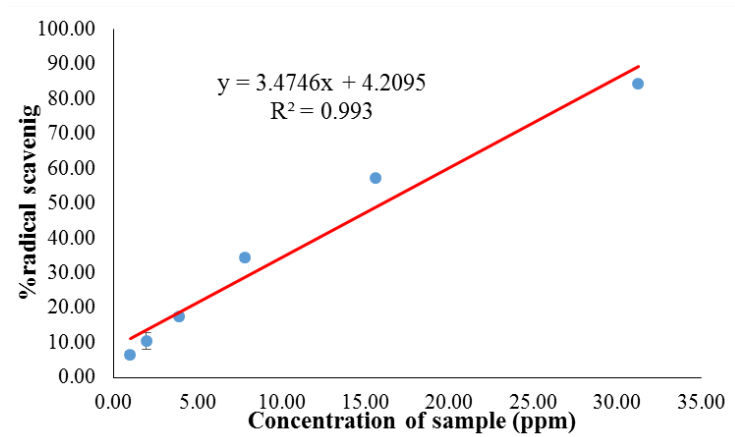


Figure A.17 Calibration curve of 15 days fermented leaves extract

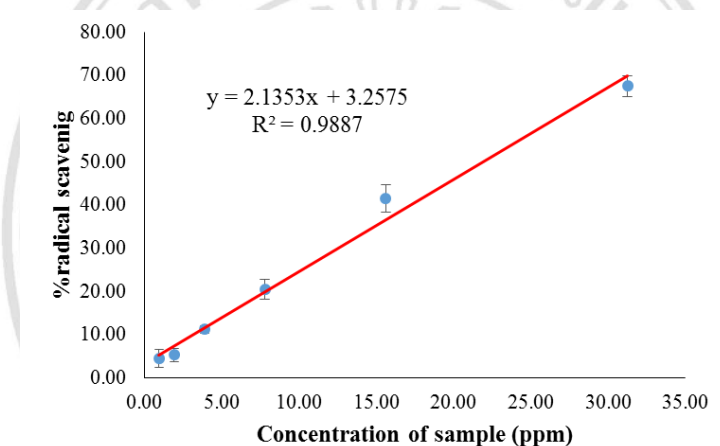


Figure A.18 Calibration curve of 30 days fermented leaves extract

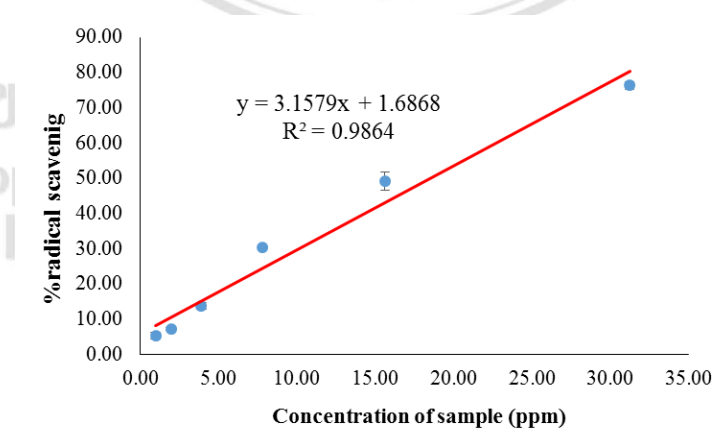


Figure A.19 Calibration curve of 45 days fermented leaves extract

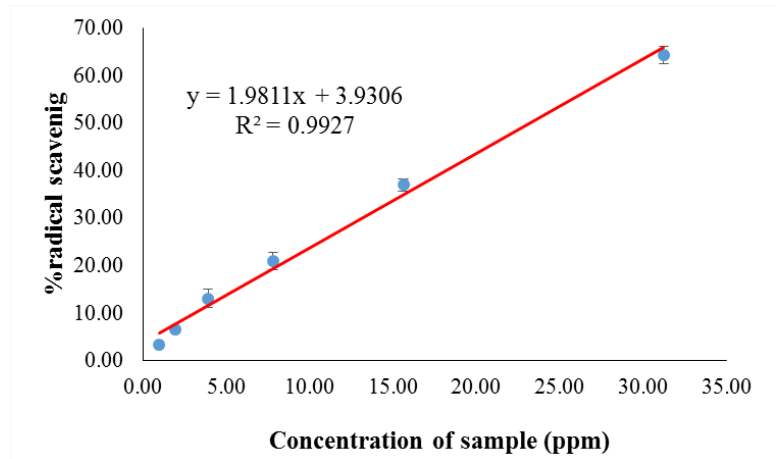


Figure A.20 Calibration curve of 60 days fermented leaves extract

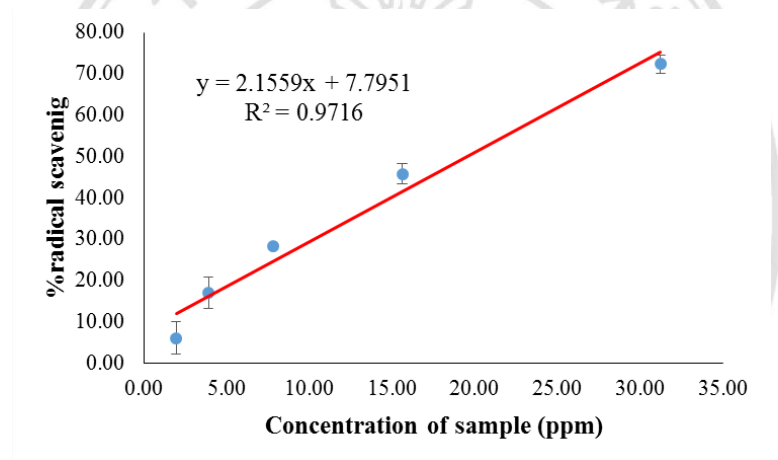


Figure A.21 Calibration curve of 90 days fermented leaves extract

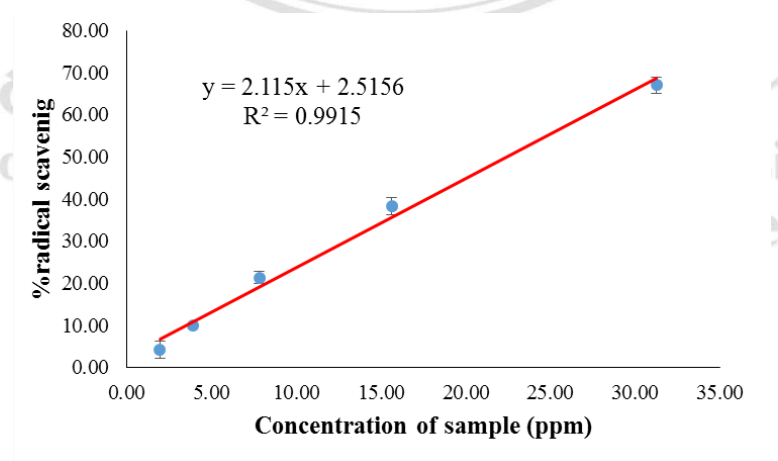


Figure A.22 Calibration curve of 120 days fermented leaves extract

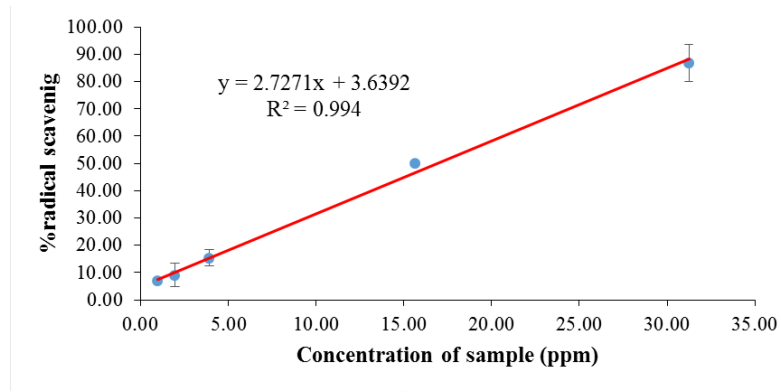


Figure A.23 Calibration curve of 150 days fermented leaves extract

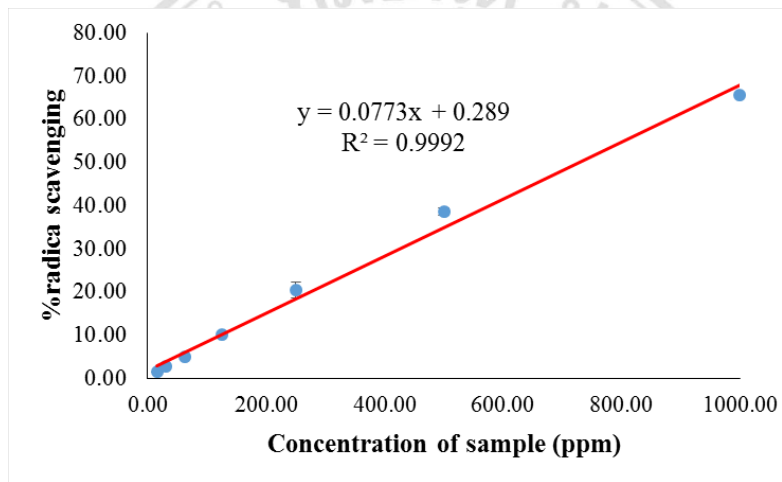


Figure A.24 Calibration curve of steamed water

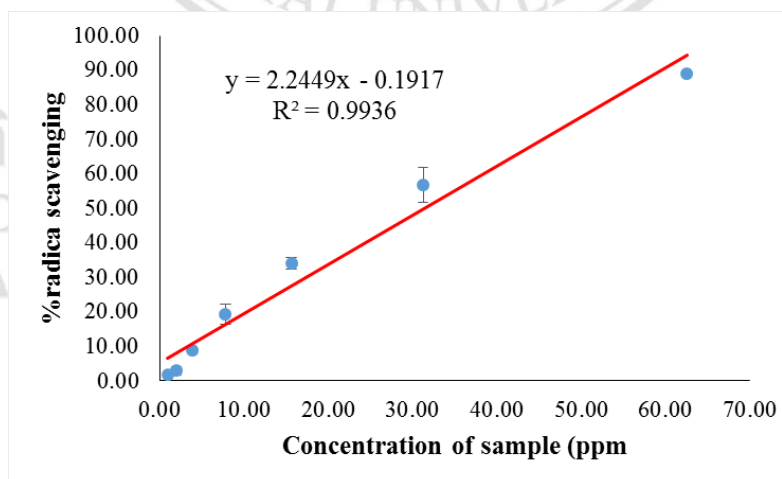


Figure A.25 Calibration curve of 15 days fermented water

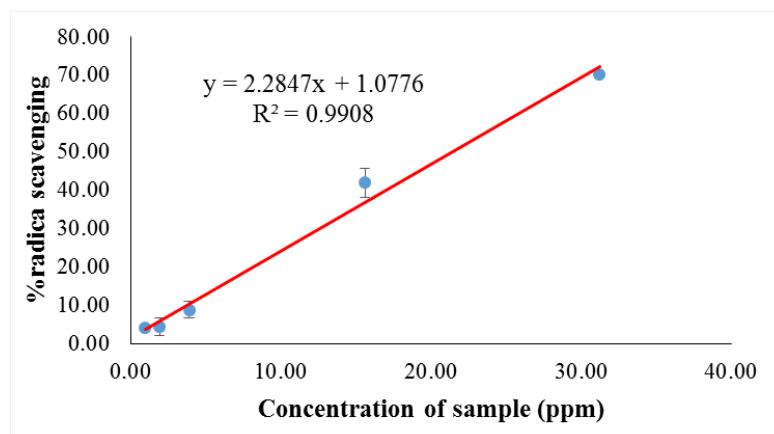


Figure A.26 Calibration curve of 30 days fermented water

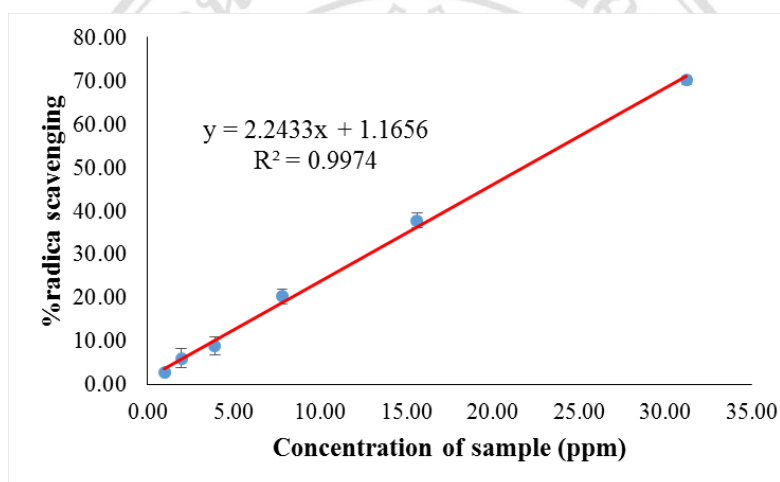


Figure A.27 Calibration curve of 45 days fermented water

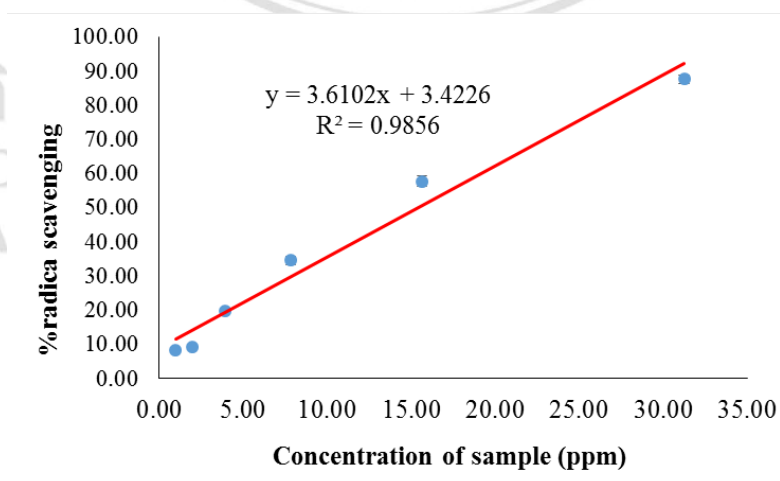


Figure A.28 Calibration curve of 60 days fermented water

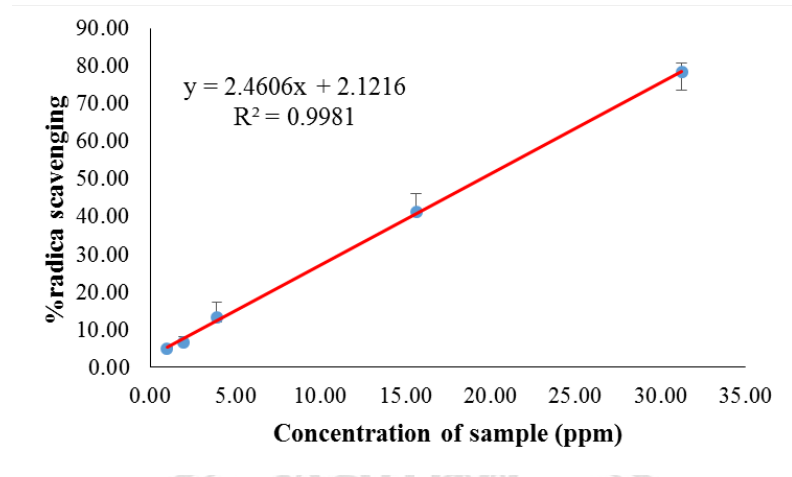


Figure A.29 Calibration curve of 90 days fermented water

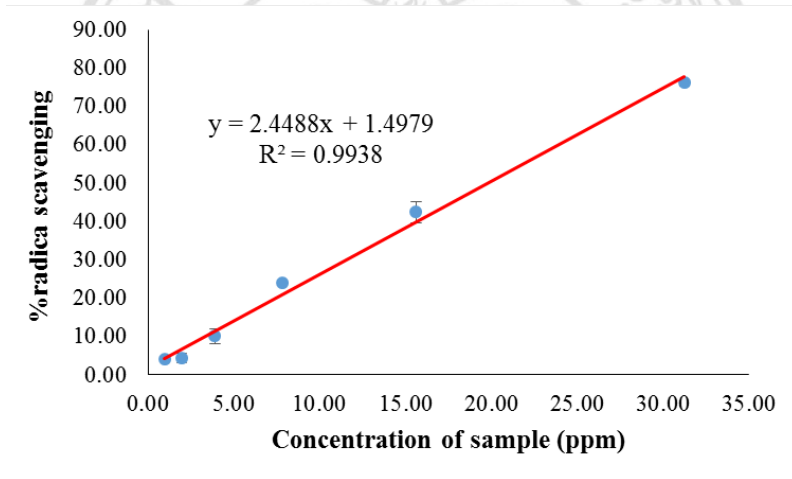


Figure A.30 Calibration curve of 120 days fermented water

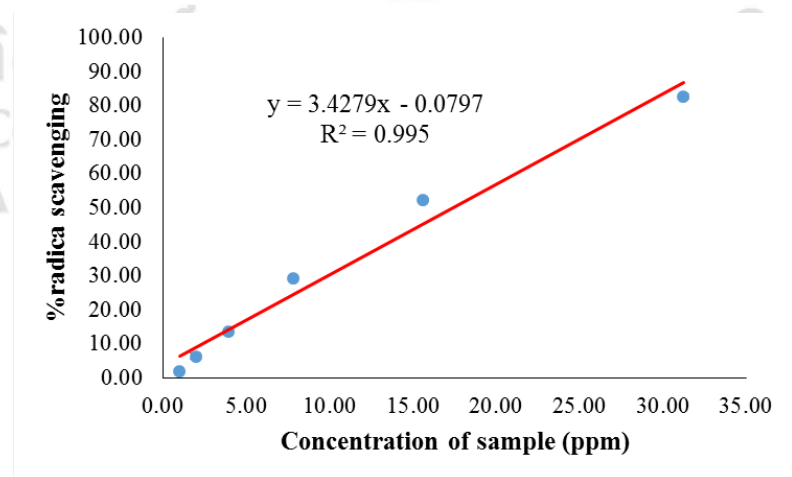


Figure A.31 Calibration curve of 150 days fermented water

CURRICULUM VITAE

- Author's Name Ms. Pasinee Chaichana
- Date/ Year of Birth July 20, 1990
- Place of Birth Chiang Rai Province, Thailand
- Education
- High school certificate, Princess Chulabhorn's College, Chiang Rai, Chiang Rai, 2008
 - B.S. (Chemistry) degree holder from, University of Phayao, 2012
- Scholarship**
- M.S. Scholarship supported by Centre of Innovation in Chemistry (PERCH-CIC) and Graduate Research Scholarships from the Graduate School Chiang Mai University
- Publication
- Chemical Constituents and Antioxidant Activity of *Camellia sinensis* var. *assamica*, Pure and Applied Chemistry International Conference 2017 (Proceeding) 2-3 February 2017, Centra Government Complex Hotel & Convention Centre, Bangkok, Thailand



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